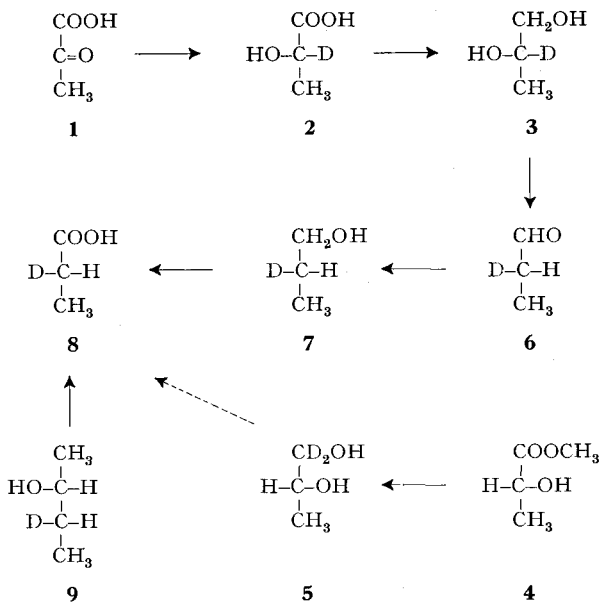
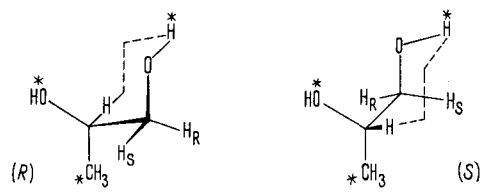
2 deuterierten chiralen Propionsäure **8**, welche eine positive normale ORD-Kurve aufwies ($[\alpha]_{500} = +1°$; $[\alpha]_{400} = +2°$; $[\alpha]_{250} = +27{,}5°$; $[\alpha]_{230} = +80°$, in Wasser)[7]. Eine Säure mit ähnlicher positiver ORD-Kurve liess sich auf gleichem Wege ausgehend von **5** erhalten.



(*S*)-2-^{2}H-Propionsäure, **8**, hergestellt in Anlehnung an frühere Arbeiten[7] durch Bromlauge-Abbau von (−)-(2*R*, 3*S*)-3-^{2}H-Butanol-(2), **9**, ist ebenfalls durch eine positive ORD-Kurve gekennzeichnet, wodurch die absolute Konfiguration des gemeinsamen Produktes **8** aus der Umsetzung von **3** und **5** als (*S*) festgelegt ist. Daraus folgt, dass sowohl bei **3** wie auch bei **5** die Wanderung des Wasserstoffatoms unter Inversion am C-2 stattfindet.



Die inzwischen erfolgte Ermittlung der Stereospezifizität von Leberalkoholdehydrogenase[9] zusammen mit den erwähnten Ergebnissen von FREY, KARABATSOS und ABELES[6] verlangt zusätzlich, dass bei (*S*)-Propandiol spezifisch das H_S-Atom, bei (*R*)-Propandiol das H_R-Atom wandert[10]. Dem sich ergebenden stereochemischen Bild kann man vorläufig durch die Annahme Rechnung tragen, dass beide enantiomeren Propandiole durch drei gleiche Haftstellen an das Enzym gebunden werden (Figur), wodurch zwischen Abgangsgruppe und wanderndem Atom eine identische sterische Beziehung eingehalten wird.

Summary. Propanedioldehydrase is shown to convert both (+)-(*S*)-2-^{2}H-propanediol, **3**, and (−)-(*R*)-1-$^2H_2$-propanediol, **5**, to specimens of deuterated propionaldehyde, for which the (*S*)-configuration has been established. Thus, in the propanedioldehydrase reaction migration of hydrogen atoms from C-1 to C-2 always occurs with inversion of configuration.

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Organisch-chemisches Laboratorium,' Eidgenössische Technische Hochschule, Zürich (Schweiz), 29. November 1965.

[7] Die Aufnahme der in dieser Arbeit erwähnten ORD-Kurven verdanken wir dem freundlichen Entgegenkommen von Dr. J. W. CORNFORTH und seinen Mitarbeitern Dr. G. RYBACK und Herrn D. T. GREEN, Milstead Laboratory of Chemical Enzymology, Sittingbourne, England.

[8] J. RÉTEY und F. LYNEN, Biochem. Z. *342*, 256 (1965).

[9] H. WEBER, A. UMANI-RONCHI und D. ARIGONI, Helv. chim. Acta *49*, (1966), im Druck.

[10] Zur Bezeichnung der zwei konfigurativ nicht identischen H-Atome vgl. [11].

[11] H. WEBER, J. SEIBL und D. ARIGONI, Helv. chim. Acta *49*, (1966), im Druck.

p-Aminobenzamidine as Inhibitor of Trypsinogen Activation

Aliphatic amino and guanidino compounds, α-keto derivatives of tyrosine, tryptophan and phenylalanine as well as the decarboxylation products of these aromatic amino acids, and also *p*-aminomethyl cyclohexane carboxylic acid and *p*-aminomethyl benzoic acid, have been shown to inhibit the activation of trypsinogen[1–7]. In the autocatalytic activation process the strongest inhibitor described so far has been 1-propylguanidine[5], while in the enterokinase-dependent reaction *p*-hydroxyphenylpyruvic acid and indolepyruvic acid have been most effective[3]. Study of the inhibitory compounds has been of interest for several reasons. Investigation of the structural requirements for inhibitors will allow a comparison of the affinities of the active sites of trypsin and enterokinase. Furthermore, several of the inhibitory agents, such as serotonin and *p*-hydroxyphenylpyruvic acid, are found in tissues in normal or diseased states, and interference with the action or activation of proteolytic enzymes may explain part of their physiological effects. Finally, potent inhibitors of trypsinogen activation should be useful in animal experiments to settle the long-standing controversy whether autocatalytic activation plays any role in the development of the catastrophic autodigestive process of acute pancreatitis.

[1] J. D. GERATZ, Biochim. biophys. Acta *56*, 599 (1962).

[2] J. D. GERATZ, Arch. Biochem. Biophys. *102*, 327 (1963).

[3] J. D. GERATZ, Arch. Biochem. Biophys. *110*, 150 (1965).

[4] J. D. GERATZ, Exper. *21*, 699 (1965).

[5] H.-J. TRETTIN and H. MIX, Hoppe-Seyler's Z. *340*, 24 (1965).

[6] A. H. C. DUBBER, G. P. MCNICOL, and A. S. DOUGLAS, Brit. J. Haemat. *11*, 237 (1965).

[7] F. MARKWARDT, H. LANDMANN, and A. HOFFMANN, Hoppe-Seyler's Z. *340*, 174 (1965).

The present communication is concerned with three new inhibitors, i.e. *p*-aminobenzamidine, 3-indole-β-ethylguanidine and β-phenylethylguanidine. The former compound was tested because it has recently been reported to be the strongest inhibitor of the tryptic hydrolysis of benzoylarginine *p*-nitroanilide[8]. The latter two compounds were examined to determine whether the presence of the guanidino group would enhance the inhibitory effectiveness beyond that of the corresponding amines which have been studied previously[4].

All inhibitor compounds were synthesized by Cyclo Chemical Corporation. The amounts of trypsin liberated in the activation mixtures were determined in an esterolytic assay employing a modification of Hestrin's colorimetric method[1] to follow the hydrolysis of the substrate *p*-toluenesulfonyl-L-arginine methyl ester. The assay mixtures were fortified with 0.1 *M* 4-guanidinobutyric acid to eliminate any further activation, a measure especially necessary to obtain unequivocal kinetic results. The phenol reagent of Folin and Ciocalteau was used to measure the products of tryptic caseinolysis.

The results in the Table reveal that *p*-aminobenzamidine is a strong inhibitor of both modes of trypsinogen activation and is considerably more effective then even 1-propylguanidine, the values for which are included in the listing. As inhibitor of enterokinase, *p*-aminobenzamidine is also superior to *p*-hydroxyphenylpyruvic acid, which has an inhibitor constant of $7.2 \cdot 10^{-4} M$[4]. A comparison of the activity of indoleethylguanidine and phenylethylguanidine with that of tryptamine and phenylethylamine[4] makes it evident that introduction of the guanidino group did not lead to any further increase in inhibitory strength. Kinetically, the three new compounds and 1-propylguanidine act as non-competitive inhibitors of trypsinogen activation as is demonstrated for *p*-aminobenzamidine in Figure 1. The non-competitive type of inhibition for the autocatalytic reaction is in accord with previous results with other inhibitors[3,4], but is in contrast to the competitive nature of the inhibitory process when benzoyl-arginine *p*-nitroanilide is the substrate for trypsin[8]. A similar dichotomous behavior has been observed with chymotrypsin where substrate derivatives exert a competitive inhibitory effect on the hydrolysis of an ester substrate, yet inhibit the caseinolytic activity in a non-competitive manner[9]. The analogy between the two enzymes could be carried even further as the inhibition of tryptic caseinolysis by *p*-aminobenzamidine also follows a non-competitive pattern (Figure 2). Conformational changes of the enzymes possibly account for the different kinetic behavior in the presence of different substrates.

Strength of inhibitors of trypsinogen activation[a]

Inhibitor	Autocatalytic activation Ki (*M*)	Activation by enterokinase
p-Aminobenzamidine	$6.7 \cdot 10^{-6}$	$2.9 \cdot 10^{-5}$
1-Propylguanidine	$8.0 \cdot 10^{-5}$	$8.3 \cdot 10^{-4}$
3-Indole-β-ethylguanidine	$8.6 \cdot 10^{-4}$	$1.3 \cdot 10^{-2}$
β-Phenylethylguanidine	$1.7 \cdot 10^{-3}$	$1.2 \cdot 10^{-2}$

[a] The mixtures for the autocatalytic reaction contained 50 μg trypsin, 1 mg trypsinogen preparation and 0.1 *M* $CaCl_2$ in 2.0 ml of 0.1 *M* tris buffer (pH 7.7). Activation was carried out for 20 min at 27°C. The mixtures with enterokinase contained 0.33 E.K.U. and 2 mg trypsinogen preparation in 2 ml of 0.02 *M* phosphate buffer (pH 5.8). Activation was for 16 min at 23°C. All enzymes were products of Mann Res. Lab., Inc.

Finally, it is of interest that, as with other enzymes, K_i values for inhibitors of trypsin vary for different substrates. Thus the inhibitor constant for *p*-aminobenzamidine in the caseinolytic reaction was determined to be $4.55 \cdot 10^{-4} M$, while the K_i value for the autocatalytic activation, as seen above, is much smaller and compares closely with the constant reported for the hydrolysis of benzoyl-arginine *p*-nitroanilide ($8.25 \cdot 10^{-6} M$ according to MARES-GUIA and SHAW[8]).

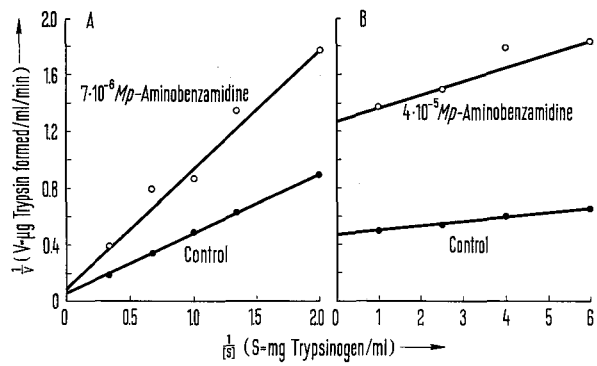

Fig. 1. Plots according to Lineweaver and Burk demonstrating that *p*-aminobenzamidine is a non-competitive inhibitor of the autocatalytic activation of trypsinogen (A) as well as of the activation by enterokinase (B). The composition of the activation mixtures was as given in the footnote of the Table except for the necessary variation in the amount of trypsinogen.

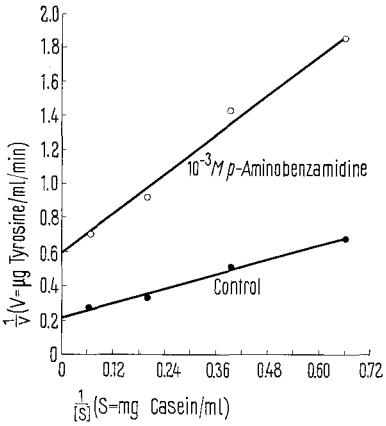

Fig. 2. Plots according to Lineweaver and Burk showing that *p*-aminobenzamidine inhibits tryptic caseinolysis in a non-competitive manner. The reaction mixtures of 3.0 ml contained 10 μg trypsin and varying amounts of casein in 0.1 *M* tris buffer (pH 7.6). Incubation was carried out for 15 min at 37°C.

Zusammenfassung. *p*-Aminobenzamidin ist der stärkste bis jetzt bekannte Hemmstoff der autokatalytischen Aktivierung des Trypsinogens und auch der Aktivierung durch Enterokinase. Die hochgradige Wirksamkeit der Verbindung empfiehlt sie zur Anwendung in der experimentellen Pankreatitis, um die Bedeutung der autokatalytischen Trypsinogenaktivierung für diesen Krankheitsprozess ermessen zu können.

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October 7, 1965.

[8] M. MARES-GUIA and E. SHAW, J. biol. Chem. *240*, 1579 (1965).
[9] J. D. GERATZ, Arch. Biochem. Biophys. *111*, 134 (1965).